



Fra-2 transgenic mice as a novel model of pulmonary hypertension associated with systemic sclerosis

Maurer, Britta ; Reich, Nicole ; Juengel, Astrid ; Kriegsmann, Jörg ; Gay, Renate E ; Schett, Georg ; Michel, Beat A ; Gay, Steffen ; Distler, Jörg H W ; Distler, Oliver

Abstract: **OBJECTIVE:** Systemic sclerosis-associated pulmonary arterial hypertension differs from idiopathic pulmonary arterial hypertension with respect to histopathology, treatment responses and survival. Medical progress on PAH is hampered by the lack of human biosamples and suitable animal models. In this study, the authors evaluated fos-related antigen 2 (Fra-2) transgenic mice as a novel model for systemic sclerosis-associated pulmonary arterial hypertension. **METHODS:** Lung sections of Fra-2 transgenic (n=12) and wild-type mice (n=6) were analysed at 16 weeks by histology using Dana Point criteria. Cellular and molecular key players were assessed by immunohistochemistry. To test the model's sensitivity to change over treatment, a subgroup of Fra-2 transgenic mice (n=6) was treated with the tyrosine kinase inhibitor nilotinib twice daily 37.5 mg orally from 8 weeks of age. **RESULTS:** Fra-2 transgenic mice developed severe vascular remodelling of pulmonary arteries and non-specific interstitial pneumonia-like interstitial lung disease resembling human systemic sclerosis-associated pulmonary hypertension. Histological features typical for systemic sclerosis-associated pulmonary arterial hypertension, such as intimal thickening with concentric laminar lesions, medial hypertrophy, perivascular inflammatory infiltrates, adventitial fibrosis, but not pulmonary occlusive venopathy were frequently detected. Platelet-derived growth factor signalling pathways were activated in pulmonary vessels of Fra-2 transgenic compared with wild-type mice. Since treatment with nilotinib strongly prevented the development of proliferative vasculopathy and lung fibrosis, the model proved to be sensitive to treatment. **CONCLUSIONS:** This study suggests that Fra-2 transgenic mice as an animal model of systemic sclerosis-associated pulmonary arterial hypertension display main characteristic features of the human disease. It therefore allows studying pathophysiological aspects and might serve as a preclinical model for interventional proof-of-concept studies.

DOI: <https://doi.org/10.1136/annrheumdis-2011-200940>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-62507>

Journal Article

Originally published at:

Maurer, Britta; Reich, Nicole; Juengel, Astrid; Kriegsmann, Jörg; Gay, Renate E; Schett, Georg; Michel, Beat A; Gay, Steffen; Distler, Jörg H W; Distler, Oliver (2012). Fra-2 transgenic mice as a novel model of pulmonary hypertension associated with systemic sclerosis. *Annals of the Rheumatic Diseases*, 71(8):1382-1387.

DOI: <https://doi.org/10.1136/annrheumdis-2011-200940>

Fra-2 transgenic mice as a novel model of pulmonary hypertension associated with systemic sclerosis

Britta Maurer¹, Nicole Reich², Astrid Jüngel¹, Jörg Kriegsmann³, Renate E. Gay¹,
Georg Schett², Beat A. Michel¹, Steffen Gay¹, Jörg HW Distler², Oliver Distler¹

¹Center of Experimental Rheumatology, University Hospital Zurich/Zurich Center of Integrative Human Physiology (ZIHP), Switzerland

²Department of Internal Medicine 3, University of Erlangen-Nuremberg, Germany

³Center for Histology, Cytology and Molecular Diagnostic, Trier, Germany

Address correspondence and reprint requests to

Oliver Distler, MD, Center of Experimental Rheumatology, University Hospital Zurich, Gloriastrasse 25, CH-8091 Zurich, Switzerland. Phone: +41-44-2552977, Fax: +41-44-2554170, E-Mail: oliver.distler@usz.ch

Key words

SSc-associated lung disease – animal model – pulmonary arterial hypertension – lung fibrosis

Total word count of the manuscript: 2445 words (excluding title page, abstract, references, figures and tables)

Abstract

Objectives

Systemic sclerosis-associated pulmonary arterial hypertension differs from idiopathic pulmonary arterial hypertension regarding histopathology, treatment responses and survival. Progress is hampered by the lack of human biosamples and suitable animal models. Herein, we evaluated Fra-2 transgenic mice as a novel model for systemic sclerosis-associated pulmonary arterial hypertension.

Methods

Lung sections of Fra-2 transgenic (n=12) and wildtype mice (n=6) were analyzed at 16 weeks by histology using Dana Point criteria. Cellular and molecular key players were assessed by immunohistochemistry. To test the model's sensitivity to change over treatment, a subgroup of Fra-2 transgenic mice (n=6) was treated with the tyrosine kinase inhibitor nilotinib at 2x 37.5mg/d p. o. from 8 weeks of age.

Results

Fra-2 transgenic mice developed severe vascular remodeling of pulmonary arteries and nonspecific interstitial pneumonia-like interstitial lung disease resembling human systemic sclerosis-associated pulmonary hypertension. Histological features typical for systemic sclerosis-associated pulmonary arterial hypertension such as intimal thickening with concentric laminar lesions, medial hypertrophy, perivascular inflammatory infiltrates, adventitial fibrosis, but not pulmonary occlusive venopathy were frequently detected. Platelet-derived growth factor signaling pathways were activated in pulmonary vessels of Fra-2 transgenic compared to wildtype mice. Since treatment with nilotinib strongly prevented the development of proliferative vasculopathy and lung fibrosis, the model proved to be sensitive to treatment.

Conclusions

Our study suggests Fra-2 transgenic mice as an animal model of systemic sclerosis-associated pulmonary arterial hypertension that displays main characteristic features of the human disease. It therefore allows studying pathophysiological aspects and might serve as a preclinical model for interventional proof of concept studies.

To date, pulmonary arterial hypertension (PAH) and interstitial lung disease (ILD) account for more than 60% of the SSc-related mortality (1).

Patients with SSc-PAH have a worse prognosis and response to PAH-specific therapies than patients with idiopathic PAH (IPAH) or PAH related to other connective tissue diseases (2-4). The observed differences in SSc-PAH and the frequent co-existence of ILD (5) support the concept of a specific pathophysiology of SSc-related pulmonary hypertension (PH), and two recent studies suggested remarkable histopathological differences of the vascular lesions associated with IPAH and those related to SSc-PAH (6, 7).

Since research is hampered by the lack of human biosamples, animal models are of outmost importance to 1) study pathophysiological interactions between the different lung manifestations, to 2) identify molecular key players and potential therapeutic targets and 3) for preclinical proof of concept studies. Unfortunately, there is a shortage of validated animal models of SSc that simultaneously display features of ILD and pulmonary vasculopathy (8), whereas established animal models of PAH rather reflect features of human IPAH than SSc-PAH (9).

The Fra-2 (Fos-related antigen-2) transgenic (tg) mouse model combines vasculopathy with fibrosis of the skin and internal organs, and Fra-2 protein is overexpressed in the skin and lungs of patients with SSc (10, 11). The aim of our study was to analyze Fra-2 tg mice as a potential animal model of SSc-PH (1) by exploring whether it displays the histopathological features considered specific for SSc-PAH, (2) by characterizing key cellular and molecular pathways contributing to the SSc-PAH specific features and (3) by assessing its sensitivity to change over treatment.

Materials and Methods

Additional information on methods is provided in the online Data Supplement.

Animals

A subgroup of Fra-2 tg mice (10, 11) (n=6) was treated with nilotinib 2x37.5 mg/d and compared to vehicle-treated Fra-2 tg mice and wildtype (wt) littermates (n=6 each). Fra-2 tg mice were backcrossed from a mixed (C57BL/6xCBA) genetic background on a pure C57/Bl6 background for at least six generations (11).

Histology

Lung sections were prepared as described previously (12). Sections were stained with hematoxylin and eosin (HE) and Masson's trichrome staining according to standard protocols.

Immunohistochemistry

For primary and secondary antibodies refer to data supplement.

Analysis of histological and immunohistochemical stainings

All slides were analyzed by two blinded independent examiners. Pictures were taken with a digital camera on an Imager1 microscope (Carl-Zeiss AG, Feldbach, Switzerland), using AxioVision software Release 4.6.

Pulmonary histopathology was assessed by microscopic criteria including pattern and distribution of inflammation, deposition of extracellular matrix, and architectural changes (13). Vascular histopathology was analyzed according to the Dana point consensus criteria (14).

Vascular remodeling was assessed by calculating the median_(Q1,Q3) of vessel wall thickness and the percentage of luminal occlusion of pulmonary arteries using GraphPad Prism software.

Cellular key players were identified by double staining with PCNA as proliferation marker and the respective cell markers for vascular smooth muscle cells (α -SMA+SM22 α +) and myofibroblasts (α -SMA+). Manually counted positive nuclei within vessel walls were confirmed independently by automated counting using image analysis software (Image J, NIH).

To assess differences in the expression of platelet-derived growth factor-BB and p(phosphorylated)-PDGFR β , positively stained vascular cells were analyzed by automated analysis of staining intensity (0=no, 1=weak, 2=moderate, and 3=intensive staining) using Image J. The median_(Q1,Q3) of staining intensity was calculated using Graph Pad Prism software.

For the analysis of inflammatory infiltrates, stains for T-cells (CD3+) and murine macrophages (F4/80+) as well as double stainings with PDGF-BB/p-PDGFR β were performed.

Statistical analysis

Nonparametric nonrelated data were analyzed with the Mann-Whitney-U test and expressed as median_(Q1,Q3). P-values<0.05 were considered statistically significant. Power calculation was performed using STATA 10.0 (StataCorp, College Station, Texas).

Results

Pulmonary pathology of Fra-2 tg mice resembles changes in SSc-PH

Pulmonary vasculopathy

To investigate Fra-2 tg mice as a potential model for SSc-PAH, we analyzed features of pulmonary vascular remodeling.

Increase in wall thickness and occlusion of pulmonary arteries were the most prominent features of pulmonary pathology in Fra-2 tg (Fig.1B) compared to wt mice (Fig.1A). Semiquantitative analysis of vascular remodeling showed that in Fra-2 tg mice the thickness of vessel walls was strongly increased compared to wt mice (median_(Q1,Q3) 44_(32,59)µm vs. 21.5_(13,36)µm; p<0.0001) (Fig.1C). Obliterated vessels were almost undetectable in wt mice (0_(0,1)%), whereas the percentage of obliterated vessels was increased to 33_(33,53)% in Fra-2 tg mice (p=0.04) (Fig.1D).

We next evaluated the histopathology of pulmonary vessels according to the DANA point criteria (14) (table 1).

Table 1: Pathologic characteristics of vasculopathies in pulmonary hypertension

	Definition according to Dana point (adapted from (14))	Presence in Fra-2 tg mice	Changes characteristic for human SSc-PAH (6, 7)	Changes characteristic for human IPAH (6, 7)
Concentric laminar intimal thickening	expansion of (myo-)fibroblasts, smooth muscle cells (SMCs)	+++ (Fig.1E)	+++	(+)
Concentric and eccentric nonlaminar thickening	predominantly composed of fibroblasts/connective tissue	+ (Figs.1F-G)	+	+
Adventitial thickening		++ (Fig.1F)	++	++
Medial hypertrophy	increase due to hypertrophy/plasia of SMCs, to an increase in connective tissue and elastic fibers, extension of SMCs into non-muscularized arteries	+ (Fig.1H)	+	+
Plexiform lesion	focal proliferation of endothelial channels lined by myofibroblasts, SMCs, and connective tissue matrix	-	(+)	+++

Dilation lesion	thin-walled vein-like vessel distally to a plexiform lesion	-	-	+
Arteritis	rarely primary, often associated with plexiform/dilation lesions, arterial wall may be necrotic and/or be infiltrated with chronic and acute inflammatory cells	-	-	+
Perivascular inflammatory infiltrates		++ (Fig.1H)	++	+
Interstitial changes	fibrosis, inflammatory infiltrates	+++ (suppl. Figs.1B-D)	+++	(+)
Pulmonary occlusive venopathy (POV, formerly PVOD)	extensive and diffuse occlusion of pulmonary venules and veins, occluding fibrous tissue may be loose and edematous with variable cellularity or dense, sclerotic, and acellular; intimal thickening mainly involves venules and small veins, luminal occlusion can be solid or eccentric with multiple lumina; media may become thickened with an increase in elastic fibers and SMCs	-	++	(+)
Pulmonary microvasculopathy (PM, formerly PCH)	localized capillary proliferation that invades pulmonary interstitium, vessels, and, less commonly, airways; panlobular and patchy distribution, often associated with pulmonary hemosiderosis	-	-	-

- absent, (+) hardly ever present/not characteristic, + rarely/can be observed, ++ often/common feature, +++ very often/characteristic feature

In summary, Fra-2 tg mice developed several features that are considered more common in SSc-PAH than in IPAH. In contrast to human SSc-PH, pulmonary occlusive venopathy (POV, formerly PVOD) was not detectable in Fra-2 tg mice.

Interstitial lung disease

Given the frequent co-existence of ILD, we additionally examined the histology of interstitial lung changes (for details refer to data supplement).

In summary, Fra-2 tg mice developed a severe interstitial pneumonitis and fibrosis with concomitant lung emphysema (suppl. Fig.1) which closely resembled the features of human nonspecific interstitial pneumonia (NSIP), which is the main histological pattern in SSc-ILD (15). In contrast to a previous study (10), honeycombing, severe scarring or other pulmonary changes resembling human UIP were not observed which might be due to the different genetic background of the Fra-2 tg mice.

Cellular composition of intimal lesions

We next characterized the cells driving the intimal thickening in Fra-2 tg mice. In wt mice, α -SMA (smooth muscle antigen) staining indicated muscular pulmonary vessels (Fig.2A). α -SMA⁺ myofibroblasts/vascular smooth muscle cells (VSMCs) (Fig.2B) were the most abundant cells within the neointima, whereas expansion of endothelial cells (van Willebrand factor (vWF)⁺) was not observed (Fig.2B). To differentiate between VSMCs and myofibroblasts, we performed additional stainings with antibodies against the VSMC-specific marker SM22 α (smooth muscle 22 α) (Figs.2C+D). In wt mice, VSMCs were mainly found within the walls of bronchi (Fig.2C), in Fra-2 tg mice, mainly in the media but not the intima of pulmonary vessels and in bronchiolar walls (Fig.2D).

The proliferation marker PCNA (proliferating cell nuclear antigen) (Figs.2E+F) was abundantly expressed by myofibroblasts showing that the observed changes were due to proliferation and not hypertrophy of cells.

In summary, mainly myofibroblasts and to a lesser extent VSMCs were key players in the remodeling of pulmonary arteries in Fra-2 tg mice which differs from the previous finding of VSMCs as the most abundant cells. This difference might either be due to technical reasons (no additional staining with a VSMC-specific

marker such as SM22 α +) or due to the different genetic background of the Fra-2 tg mice.

Molecular mechanisms of vascular remodeling: PDGF signaling

Next, we assessed potential molecular mechanisms of both vasculopathy and ILD. In patients with IPAH, PDGF-BB has been proposed as a novel therapeutic target (16, 17), and a recent study reported a higher immunoreactivity for p-PDGFR β in SSc-PAH compared to IPAH (18).

Thus, we analyzed the expression of PDGF-BB and the phosphorylated (=activated) PDGFR β in the lungs of Fra-2 tg and wt mice by assessing the staining intensity. Both, the expression of PDGF-BB (Fig.3B) and of p-PDGFR β (Fig.3E) was increased in vascular structures of Fra-2 tg compared to wt mice (Figs.3A+D). Occasionally, increased staining for p-PDGFR β could be observed in the alveolar epithelium of Fra-2 tg mice (Fig.3E). Semiquantitative analysis confirmed the substantially increased expression of PDGF-BB in pulmonary vessels in Fra-2 tg compared to wt mice (median_(Q1,Q3) of staining intensity 3_(2.8,3.0) vs. 0_(0,1); p<0.0001) (Fig.3C). A similarly strong upregulation was observed for p-PDGFR β (3_(2,3) vs. 0_(0.0,0.3); p<0.0001) (Fig.3F). In addition to vascular structures, tissue macrophages (F4/80+) in Fra-2 tg mice showed a strong upregulation of both PDGF-BB (Fig.3G) and p-PDGFR β (Fig.3H). These findings suggest a potential role for PDGF-BB in the pulmonary pathophysiology of Fra-2 tg mice.

Nilotinib prevents vascular remodeling and fibrosis in Fra-2 tg mice

Next we evaluated whether targeting the PDGF-BB/PDGFR pathway using the tyrosine kinase inhibitor nilotinib could modify the vascular and fibrotic lesions in Fra-2 tg mice.

Pulmonary vasculopathy

Treatment with nilotinib strongly prevented the remodeling of pulmonary arteries (Fig.4B) compared to vehicle-treated Fra-2 tg mice (Fig.4A). The endothelial cell layer (vWF+) was not affected upon treatment with nilotinib (Fig.4B) compared to wt mice (Fig.4A). Semiquantitative analysis showed that compared to vehicle-treated Fra-2 tg mice, in nilotinib treated Fra-2 tg mice both the thickness of vessel walls (median_(Q1,Q3) 44_(32,59) vs. 22.5_(18,28)µm, p<0.0001) (Fig.4C) and the percentage of obliterated vessels (33_(13,53)% vs. 0_(0,7)%, p=0.03) (Fig.4D) was significantly reduced reaching the levels of wt mice.

Treatment with nilotinib resulted in a reduced proliferation of α-SMA+ cells compared to vehicle-treated Fra-2 tg mice as analyzed by PCNA-double-staining (Figs.4F+E). These findings were confirmed by semiquantitative analysis with reduction of proliferating (PCNA+) α-SMA+ cells in nilotinib treated compared to vehicle-treated Fra-2 tg mice (median_(Q1,Q3) 22_(19,43) vs. 87_(57,90) PCNA+ vascular cells/HPF) (Fig.4G).

As expected, the expression of p-PDGFRβ (Fig.5B), and also of PDGF-BB (Fig.5E) was reduced in vascular structures compared to vehicle-treated mice (Figs.5A+D) showing successful targeting of PPDGFR. Semiquantitative analysis demonstrated a reduced staining intensity for p-PDGFRβ (Fig.5C) and also for PDGF-BB (Fig.5F) in the pulmonary vessels of nilotinib treated compared to vehicle-treated Fra-2 tg mice (median_(Q1,Q3) 2_(2.0,2.3) vs. 3_(2.8,3.0), p=0.03; 1_(0.8,1.0) vs. 3_(2,3), p=0.004).

Interstitial lung disease

Treatment with nilotinib also inhibited the development of lung fibrosis in Fra-2 tg mice when assessing the accumulation of connective tissue by Masson's trichrome staining (Figs.5G+H).

Interestingly, treatment with nilotinib diminished the numbers of macrophages (Fig.5J, F4/80+), whereas T cells (Fig.5J, CD3+) were not affected compared to vehicle-treated Fra-2 tg mice (Fig.5K). However, there was a considerable heterogeneity between mice in the effects of nilotinib on inflammatory cells, and the differences to vehicle-treated mice did therefore not reach statistical significance in the semiquantitative analysis.

Discussion

To date, there is substantial evidence on several levels that SSc-PAH, though sharing similarities, substantially differs from IPAH and thus might warrant different therapeutic approaches. However, the development of targeted therapies is hampered by the lack of human biosamples of SSc patients and by the lack of suitable animal models for preclinical studies.

Our study suggests Fra-2 tg mice as a potential model for SSc-PAH. The comprehensive histological and immunohistochemical analysis showed that Fra-2 tg mice displayed many pathologic changes characteristic for the vascular remodeling in human SSc-PAH (6, 7, 18). The model can however not be used to further delineate mechanisms underlying POV, as POV-specific features were not identified in our study. Besides vasculopathy, interstitial inflammation and fibrosis closely resembling human NSIP as the most common form of SSc-ILD could be observed. Fibroblastic foci and honeycombing, associated with UIP, were rarely detectable in our study, but were identified more frequently in a recent study (10). This might be explained by the different background of Fra-2 tg mice, as the mice in this study were backcrossed from a mixed (C57BL/6×CBA) genetic background on a pure C57/Bl6 background.

However, besides allowing the evaluation of pathophysiologic mechanisms and in particular the interplay of pro-fibrotic and vascular processes, Fra-2 tg mice might also serve as a preclinical model for interventional proof of concept studies. The tyrosine kinase inhibitor nilotinib by blocking PDGFR signaling prevented the development of both proliferative pulmonary vasculopathy and fibrosis in Fra-2 tg mice which argues for SSc-specific therapies of PH. Furthermore, our data support previous studies on PDGF as key mediator of vasculopathy in SSc-P(A)H (17-19). Inhibition of PDGF signaling, e. g. by tyrosine kinase inhibitors, might be an example

of a promising future therapy for P(A)H (20) with particular evidence in SSc-PAH, as it targets vascular as well as fibrotic changes (21, 22).

The role of Fra-2 as a potential key player for the development of (pulmonary) vasculopathy and fibrosis in SSc should be addressed in further studies. Recent data demonstrated an increased expression of Fra-2 in lung biopsies from patients with UIP, NSIP and SSc-associated NSIP (10). Based on previous data on microvasculopathy and dermal fibrosis in SSc patients (11, 23), a recent study demonstrated substantial anti-fibrotic effects of AP-1 inhibition in different animal models of SSc (24). Thus, Fra-2/AP-1 itself might represent an interesting molecular target for future SSc-specific therapies, even more so, since several pro-inflammatory chemokines and cytokines implicated in the pathogenesis of SSc were expressed in high levels in the lungs of Fra-2 tg mice including CXCL5, CCL2, IL-2, IL-4, IL-6 (10). Inhibition of these downstream targets with currently available drugs might elucidate further treatment options. AP-1 is induced by growth factors, cytokines and oncoproteins which are involved in the proliferation, survival, differentiation and transformation of cells (25). Whether Fra-2 related pathogenic changes in vivo might be accelerated by other factors such as pro-inflammatory stimuli or hypoxia (26) in pulmonary disease will have to be addressed in future studies.

Taken together, the model of Fra-2 tg mice is the first animal model that simultaneously displays major histopathological features of pulmonary SSc, e. g. pulmonary proliferative vasculopathy and interstitial fibrosis, which occur frequently together in patients with SSc and are associated with a high mortality. Fra-2 tg mice hold great promise to further delineate the pathophysiological links between vascular remodeling and fibrosis in pulmonary SSc and to identify potential specific molecular and cellular targets for intervention. This study also underlines a prominent role of

PDGF in the pathophysiology of pulmonary SSc and suggests Fra-2 tg mice as a preclinical model for interventional proof of concept studies in pulmonary SSc.

Licence for Publication

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in ARD and any other BMJ PGL products and sublicences such use and exploit all subsidiary rights, as set out in our licence (<http://group.bmj.com/products/journals/instructions-for-authors/licence-forms>).

Competing Interests

J.H.W. Distler has consultancy relationships and/or has received research funding from Boehringer Ingelheim, Celgene, Bayer Pharma, Actelion, Pfizer, Ergonex, BMS, JB Therapeutics, Anaphore, Inc, Sanofi-Aventis, Novartis, Array Biopharma and Active Biotech in the area of potential treatments of scleroderma and is stock owner of 4D Science.

O. Distler has consultancy relationship and/or has received research funding from Actelion, Pfizer, Ergonex, BMS, Sanofi-Aventis, United BioSource Corporation, medac in the area of potential treatments of scleroderma and its complications. He has received lecture honoraria from Actelion, Pfizer, Encysive and Ergonex. The real or perceived potential conflicts listed above are accurately stated.

All others were supported by their respective institutions.

References

1. Steen VD, Medsger TA. Changes in causes of death in systemic sclerosis, 1972-2002. *Ann Rheum Dis* 2007;66:940-4.
2. Channick RN, Simonneau G, Sitbon O, et al. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. *Lancet* 2001;358:1119-23.
3. Condliffe R, Kiely DG, Peacock AJ, et al. Connective Tissue Disease-associated Pulmonary Arterial Hypertension in the Modern Treatment Era. *Am J Respir Crit Care Med* 2009;179:151-7.
4. Girgis RE, Mathai SC, Krishnan JA, et al. Long-term outcome of bosentan treatment in idiopathic pulmonary arterial hypertension and pulmonary arterial hypertension associated with the scleroderma spectrum of diseases. *J Heart Lung Transplant* 2005;24:1626-31.
5. Vonk MC, Broers B, Heijdra YF, et al. Systemic sclerosis and its pulmonary complications in The Netherlands: an epidemiological study. *Ann Rheum Dis* 2009;68:961-5.
6. Dorfmueller P, Humbert M, Perros F, et al. Fibrous remodeling of the pulmonary venous system in pulmonary arterial hypertension associated with connective tissue diseases. *Human Pathol* 2007;38:893-902.
7. Overbeek MJ, Vonk MC, Boonstra A, et al. Pulmonary arterial hypertension in limited cutaneous systemic sclerosis: a distinctive vasculopathy. *Eur Respir J* 2009;34:371-9.
8. Derrett-Smith EC, Denton C, Sonnylal S. Animal models of scleroderma: lessons from transgenic and knockout mice. *Curr Opin Rheumatology* 2009;21:630-5.

9. Stenmark KR, Meyrick B, Galie N, et al. Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L1013-32.
10. Eferl R, Hasselblatt P, Rath M, et al. Development of pulmonary fibrosis through a pathway involving the transcription factor Fra-2/AP-1. *Proc Natl Acad Sci* 2008;105:10525-30.
11. Maurer B, Busch N, Jungel A, et al. Transcription Factor Fos-Related Antigen-2 Induces Progressive Peripheral Vasculopathy in Mice Closely Resembling Human Systemic Sclerosis. *Circulation* 2009;120:2367-76.
12. Distler JHW, Jüngel A, Huber LC, et al. Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis. *Arthritis Rheum* 2007;56:311-22.
13. Myers JL, Katzenstein AL. Beyond a consensus classification for idiopathic interstitial pneumonias: progress and controversies. *Histopathol* 2009;54:90-103.
14. Pietra GG, Capron F, Stewart S, et al. Pathologic assessment of vasculopathies in pulmonary hypertension. *J Am Coll Cardiol* 2004;43:25S-32S.
15. Bouros D, Wells AU, Nicholson et al. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. *Am J Respir Crit Care Med* 2002;165:1581-6.
16. Humbert M, Monti G, Fartoukh M, et al. Platelet-derived growth factor expression in primary pulmonary hypertension: comparison of HIV seropositive and HIV seronegative patients. *Eur Respir J* 1998;11:554-9.
17. Perros F, Montani D, Dorfmüller P, et al. Platelet-derived Growth Factor Expression and Function in Idiopathic Pulmonary Arterial Hypertension. *Am J Respir Crit Care Med* 2008;178:81-8.

18. Overbeek MJ, Boonstra A, Voskuyl AE, et al. Platelet-derived growth factor receptor-beta and epidermal growth factor receptor in pulmonary vasculature of systemic sclerosis-associated pulmonary arterial hypertension versus idiopathic pulmonary arterial hypertension and pulmonary veno-occlusive disease: a case-control study. *Arthritis Res Ther* 2011;13:R61.
19. Schermuly RT, Dony E, Ghofrani HA, et al. Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest* 2005;115:2811-21.
20. Souza R, Sitbon O, Parent F, et al. Long term imatinib treatment in pulmonary arterial hypertension. *Thorax* 2006;61:736.
21. Spiera RF, Gordon JK, Mersten JN, et al. Imatinib mesylate (Gleevec) in the treatment of diffuse cutaneous systemic sclerosis: results of a 1-year, phase IIa, single-arm, open-label clinical trial. *Ann Rheum Dis* 2011;70:1003-9.
22. Distler O, Distler J, Varga J, et al. A multi-center, open-label, proof of concept study of imatinib mesylate demonstrates no benefit for the treatment of fibrosis in patients with early, diffuse systemic sclerosis (abstract). *Arthritis Rheum* 2010;70:S233-4.
23. Reich N, Maurer B, Akhmetshina A, et al. The transcription factor Fra-2 regulates the production of extracellular matrix in systemic sclerosis. *Arthritis Rheum* 2010;62:280-90.
24. Avouac J, Palumbo K, Tomcik M, et al. The transcription factor AP-1 mediates the pro-fibrotic effects of TGFbeta and contributes to the development of experimental dermal fibrosis. *Ann Rheum Dis* published online first: 2 December 2011. doi:10.1002/art.33501.
25. Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta*. 1991;1072:129-57.

26. Butler TL, Pennypacker KR. The transcriptional response to hypoxic insult controlled by FRA-2. *Gene Expr.* 2005;12:61-7.

Figure legends

Fig. 1 Pulmonary pathology of Fra-2 tg mice: vascular remodeling

(A) Pulmonary histology of wt mice (HE staining, arrow indicates vascular structure, B indicates bronchus). (B) Remodeling of pulmonary vessels with luminal narrowing and obliteration in Fra-2 tg mice (arrows; HE staining, B indicates bronchi). Figures C+D show the semiquantitative analysis with evaluation of vessel wall thickness and luminal obliteration in Fra-2 tg compared to wt mice. (E-H) illustrate the main pathologic changes of pulmonary vessels in Fra-2 tg mice: concentric laminar lesion due to formation of neointima by myofibroblasts and VSMCs (E, α -SMA positive, blue, short arrows) whereas the endothelial cell layer (vWF positive, red, long arrows) remained unaffected; (F) medial hypertrophy (arrow, HE staining) and perivascular inflammatory infiltrates (white asterisk), (G) adventitial fibrosis (Masson's trichrome stain, bold arrow) and concentric laminar lesion (long arrow), and (H) eccentric nonlaminar lesion (Masson's trichrome stain, arrow) due to expansion of fibroblasts and deposition of extracellular matrix proteins. Pictures are representative examples of 6 wt and 6 Fra-2 tg mice. Data are expressed as median and interquartile range. * indicates p-values <0.05

Fig. 2 Cellular key players of vascular remodeling in Fra-2 tg mice

Compared to wt mice (A), severe vascular remodeling of pulmonary arteries could be observed in Fra-2 tg mice (B). In Fra-2 tg mice, increase in vessel wall thickness occurred due to expansion of α -SMA positive cells (dark blue, short arrows), whereas the endothelial cell layer was not increased (vWF-positive, red, long arrows). Increase in vessel wall thickness was mainly due to myofibroblasts (α -SMA positive, SM22 α negative), whereas VSMCs (α -SMA (blue) and SM22 α (purple) positive) were mainly expressed in bronchiolar (B) walls in wt mice (C, arrows), and additionally in

the media of muscular vessels in Fra-2 tg mice (D, arrows). (E) Formation of neointima was mainly due to proliferation of α -SMA-positive cells (dark blue, short arrows) as indicated by the double staining with the proliferation marker PCNA (brown, long arrows). (F) shows the proliferating cells in a higher magnification. Pictures are representative examples of 6 wt and 6 Fra-2 tg mice.

Fig. 3 Molecular mechanisms of vascular remodeling in Fra-2 tg mice: PDGF signaling

Compared to wt mice (A), in Fra-2 tg mice (B), vascular structures (short arrows) showed an increased staining for PDGF-BB (brown, long arrows) as shown in the semiquantitative analysis (C). The same difference could be observed for p-PDGFR β (brown, long arrows; arrowhead indicates increased staining in the alveolar epithelium) in pulmonary vessels (bold arrow) of Fra-2 tg (E) compared to wt mice (D; bold arrow indicates vascular structure, arrows indicate staining in the interstitium). (F) shows the respective semiquantitative analysis. In addition, there was also an increased expression of PDGF-BB (G, brown) and its activated receptor (H, brown) in pulmonary macrophages (green, F4/80-positive, arrows). Pictures are representative examples of 6 wt and 6 Fra-2 tg mice. Data are expressed as median and interquartile range. * indicates p-values <0.05

Fig. 4 Effects of nilotinib on vascular remodeling in Fra-2 tg mice

Compared to vehicle-treated mice (A, short arrows), treatment with nilotinib strongly prevented the vascular remodeling of pulmonary arteries (B, short arrows). The endothelial cell layer was not affected (red, vWF positive cells, long arrows. (C+D) demonstrate the differences in vessel wall thickness and luminal obliteration in the semiquantitative analysis. As indicated by the double staining with the proliferation

marker PCNA (brown, long arrows) compared to vehicle-treated mice (E), the expansion of α -SMA-positive cells (blue, myofibroblasts and VSMCs, short arrows) was reduced upon treatment with nilotinib (F), additionally demonstrated in the semiquantitative analysis (G). Pictures are representative examples of 6 wt, 6 vehicle-treated and 6 nilotinib treated Fra-2 tg mice. Data are expressed as median and interquartile range. * indicates p-values <0.05

Fig. 5 Effects of nilotinib on PDGF signaling and interstitial lung disease

Compared to vehicle-treated Fra-2 tg mice (A, D), the expression of both p-PDGFR β (B, brown, long arrows) in vascular structures (short arrows) and PDGF-BB (E, brown, long arrows) was reduced by treatment with nilotinib. (C+F) show the results of the respective semiquantitative analyses. Interstitial fibrosis (appears green in Masson's trichrome staining) (H) was strongly prevented by nilotinib compared to vehicle-treated Fra-2 tg mice (G). In 3 out of 6 mice, nilotinib (J) diminished the number of macrophages (F4/80 positive, green, arrows) compared to vehicle-treated mice (I) whereas CD3 positive cells (purple) remained unaffected. Pictures are representative examples of 6 wt, 6 vehicle-treated and 6 nilotinib treated Fra-2 tg mice. Data are expressed as median and interquartile range. * indicates p-values <0.05